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CONTRIBUTION TO THE QUESTION OF THE CONTAMINATION OF SUSPENDED-SUBSTANCES FILTERS BY GERMS

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16. Abstract In this paper, we report measurements of the germ-count of HEPA-filtered air, and also determinations of the germ content of HEPA filters, in the air-conditioning plant of an experimental animal department constructed in accordance with the barrier system. 1. In the 888 samples routinely collected with slit-samplers during the 26 months' operation of the filters, a total quantity of 503 m^3 HEPA-filtered supply air was investigated. No bacteria or fungi were detected in any of the samples taken during this period. 2. Before the replacement of the HEPA filters after 26 months'operation, a total quantity of 114.9 m^3 HEPA-filtered supply air was bacterilogically investigated in another 195 measurements undertaken with slit-samplers, Greenburg-Smith impingers and sedimentation-plates. In these investigations, no germs were detected, either with slit-samplers or on sedimentation-plates. In measurements performed with Greenburg-Smith impingers on a total quantity of 20.4 m^3 air, a total of 3 colonies of Staph. albus were detected in 2 out of 36 samples, but these should be regarded as contamination produced during the test procedure itself.

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CONTRIBUTION TO THE QUESTION OF THE CONTAMINATION OF SUSPENDED-SUBSTANCES FILTERS BY GERMS

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Because of the results of bacteriological control experiments at the air-conditioning plant of our experimental animal department [14], we subsequently had installed high capacity suspended-substances filters of specialty class S (HOSCH) to obtain germ-free supply air. These are situated inside the individual animal rooms directly at the end of the supply air ducts. Since then we have routinely examined the supply air filtered with HOSCH filters for germ content, and during the time of operation of the HOSCH filters up to now we were not able to demonstrate any penetration of germs. /273

The question which has been brought up repeatedly in recent times about the possible "growing through" of germs through HOSCH filters [1,5,8,10,13,15,16,17,18,19] could not be clarified with certainty up to now since there are too few investigations at existing plants. A "growing through" of germs was described by Grun [7], for a precipitation of moisture on the filters, whereas according to the investigations of Botzenhart and Ruden [2,3] no breaking through of bacteria and fungi is

* Numbers in the margin indicate pagination of original foreign text.

to be expected when keeping within a relative humidity between 45 and 70%.

After an operation time of 26 months, we changed the HOSCH filters of the air conditioning plant of our experimental animal department and carried out bacteriological investigations on the filters in situ and on the filter medium itself; we now report on these results. /274

Experimental Animal Plant

The structural layout of the experimental animal department, as well as the construction of the air conditioning and ventilation plant and of the air filter system, has been described by us elsewhere in detail [14].

Methods of Measurement

The germ counts in air were determined by the following methods of measurement:

1. Large volume slit-sampler (C.F. Casella, London). The apparatus, working according to the impaction principle, separates the bacteria-carrying particles from a stream of air that is sucked in and strongly accelerated through one to four slits, each 0.1 to 4.45 cm large, to a nutrient medium plate of diameter 15 cm which is rotating and located underneath the slits. The rate of air flow can be varied, depending on the number of open slits, from 175,350,525 to 700 l min. In our experiments the apparatus was set up so that 350 l/min of air were measured; the collection time amounted to 2 or 3 minutes, so that 700 or 1050 liters of air were extracted per probe and plate.

2. The Greenburg-Smith-impinger [14,20], working according

to the impingement principle, was put in in addition, in order to determine the number of "viable particles". With this method volumes of air from 500-700 l per probe were measured.

3. As an additional control, sedimentation plates with a diameter of 15 cm were installed.

4. The qualitative proof of germs in the filter medium was achieved by means of a contact sampling technique. On the clean air side, agar flex bags (according to Kanz) were laid on the medium of the dismantled HOSCH filters, or pieces of filter about 7x4.5 cm large were cut out and laid on enriched nutrient media. On the dust-laden air side, strips about 7x4.5 cm were cut out and transferred to fixed nutrient media.

5. Nutrient media, incubation.

For the determination of the air germ count with split samplers, as a rule, plate count agar (DIFCO) was employed as the nutrient medium.

For catching the germs when using the Greenburg-Smith-impingers, a neutral buffered salt solution [20] was used.

As a nutrient medium for the sedimentation plates, blood agar was employed. For proof of germs by means of the contact sampling technique, agar flex bags (according to Kanz), plate count agar (DIFCO), blood agar, and trypticase soy agar (DIFCO) were used.

Upon insertion of double determinations, the air germ plates and sedimentation plates are incubated, each at half aerobic, for 5 days at 22°C and 1 day at 37°C with a 4 day post incubation period at 22°C; for triple determinations, an additional aerobic incubation of the plates was undertaken for 5 days at 30°C. The contact sampling cultures which were prepared in triple determinations were - if not noted otherwise - incubated 5 days at 37°C, 10 days at 30°C and 14 days at 22°C, aerobically and anaerobically.

The distance of the air suction nozzle of the measuring apparatus from the HOSCH filters amounted to a constant 0.3 to 0.4 cm in the probe extractions from the clean air side.

6. Measurements of the particle content of the HOSCH-filtered supply air were carried out with a scattering particle counting apparatus CLIMET CI 250 [24], which subdivides the particles in classes by size: 0.5μ, 1μ, 2μ, 5μ, 10μ and larger. The distance of the probe amounted to 0.3 to 0.4 cm for these investigations as well.

Results /275

Since installation of the HOSCH filters in our air conditioning plant, we routinely investigated bacteriologically, in 888 probe extractions, 503 cm^3 of air, during an operation time of the filters of 26 months in laboratory I and of 22 months in laboratory II, and using the slit-sampler MK II and the large volume slit-sampler.

In no instance were we able to demonstrate the existance of germs in the HOSCH-filtered air with the employed methods during this time interval.

Since the "closed division" of Laboratory I was opened after completion of a series of investigations because of maintanance and structural alteration work, we made use of the opportunity to undertake bacteriological investigations after a 26 month operation time before changing the HOSCH filters.

The results of the germ count measurements of the HOSCH-filtered supply air after an operation time of the filters of 26 months are summarized in Table 1. According to this, only 3 germs could be verified for the three biological methods of air measurement employed, only in 2 probes and only for the Greenburg-Smith-impingers in one of the three methods of cultivation; these are to be evaluated as secondary contamination. As wa have already noted and reported on earlier [14], just for this method of measurement does the danger of contamination upon analyzing the collected fluid exist.

For the other methods of investigation, bacteria or fungi could not be demonstrated in any case in the HOSCH-filtered supply air.

In measurements of the particle content of the HOSCH-filtered supply air, which were carried out in parallel determinations with the biological methods of measurement, we found particle values with the CLIMET particle counter which were better than the maximal tolerated particle count (0.5µ and larger) of 3500 particles/m^3 [23] for clean air class 100.

In Table 2 the measured germ and particle counts are contrasted. In our investigations we were under the maximal tolerated count of viable particles of 3.5 germs/m^3 of air of the "NASA Standard for Bioclean Rooms" [22], with our germ count values of 0 germs/m^3.

In the next set of experiments the filter medium itself, dust-laden air side and clean air side, was to be tested quantitatively and qualitatively for germ colonization and for possible resulting "breaking through" of microorganisms to the clean air side.

The extraction of the filter material for the bacteriological investigations was done immediately after dismantling the filters and was always completed within one hour after removal of the filters. In taking apart and spreading out the folded filter mats, the filter on the clean air side showed no discoloration or deposits, in contrast to the dirty brown discolored dust-laden air side of the filters with partial fine-grained deposits (Figures 1,2,3).

The contact sampling preparations of the dust-laden and clean air sides were carried out using the method described above. In Table 3 the values of the quantitative investigations are summarized. From this we see that for 1.5% of the probes extracted from the clean air side of the filter medium, a total of 3 germs could be demonstrated, which very likely are to be taken as contamination upon probe extraction and not as real "germ break-through", since the colonies were always found on the cut edge of the preparate. Also, additional investigations using a culture method with enriched nutrient substrates (blood agar, trypticase soy agar) did not bring any higher germ count on the clean air side and did not change the result.

TABLE 1.

MICROORGANISM COUNTS IN HEPA-FILTERED AIR AFTER 26 MONTHS' OPERATION OF FILTERS

Labor	Method of investigation	Number of probes per method of incubation	Investigated volume of air (liter) per method of incubation	Incubation 5 d, 22°C germ count	Incubation 5 d, 30°C germ count	Incubation 1 d, 37°C+ 4 d, 22°C germ count
A	impaction procedure	5	5250	0/m^3	0/m^3	0/m^3
	sedimentation plate	5	-	0/plate/h	0/plate/h	0/plate/h
	impingement procedure	2	1400	0/m^3	0/m^3	0/m^3
B	impaction procedure	5	4254	0/m^3	0/m^3	0/m^3
	sedimentation plate	5	-	0/plate/h	0/plate/h	0/plate/h
	impingement procedure	2	1000	0/m^3	0/m^3	0/m^3
C	impaction procedure	5	5250	0/m^3	0/m^3	0/m^3
	sedimentation plate	5	-	0/plate/h	0/plate/h	0/plate/h
	impingement procedure	2	1000	0/m^3	0/m^3	0/m^3
D	impaction procedure	5	5250	9/m^3	0/m^3	0/m^3
	sedimentation plate	6	-	0/plate/h	0/plate/h	0/plate/h
	impingement procedure	2	1000	0/m^3	0/m^3	1/m^3
E	impaction procedure	5	5250	0/m^3	0/m^3	0/m^3
	sedimentation plate	6	-	0/plate/h	0/plate/h	0/plate/h
	impingement procedure	2	1000	0/m^3	0/m^3	2/m3
VQ	impaction procedure	5	5250	0/m^3	0/m^3	0/m^3
	sedimentation plate	8	-	0/plate/h	0/plate/h	0/plate/h
	impingement procedure	2	1400	0/m^3	0/m^3	0/m^3
	impaction procedure	30	31500	M=0/m^3	M=0/m^3	M=0/m^3
	sedimentation plate	35	-	M=0/plate/h	M=0/plate/h	M=0/plate/h
	impingement procedure	12	6800	M=0/m^3	M=0/m^3	M=0.44/m^3

TABLE 2 /277

VIABLE AND NONVIABLE PARTICLE COUNTS IN HEPA-FILTERED AIR AFTER 26 MONTHS' OPERATION OF FILTERS. MONITORING VIABLE PARTICLES WITH CASELLA LARGE-VOLUME SLIT SAMPLER, NONVIABLE PARTICLES WITH CLIMET Cl 250

Investigated quantity of air	94500 l			12621 l		
Particle size	-	0.5μ	1μ	2μ	5μ	10μ
Germs or particles/m^3	0 germ	76 particle	5 particle	0 particle	0 particle	0 particle

Germ count determination with Casella Large volume slit-sampler, Particle count with CLIMET Cl 250.

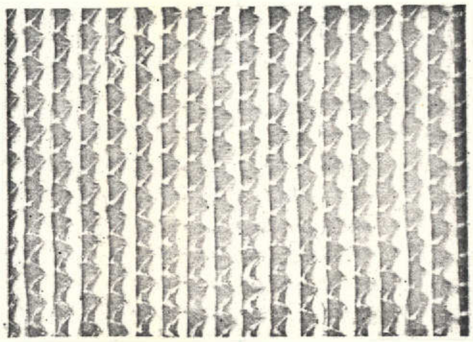

Figure 1. View of part of HEPA-filter at clean air side after 26 months' operation of filter.

Figure 2. View of part of HEPA-filter at dustladen air side after 26 months' operation of filter.

TABLE 3.

NUMBER OF COLONIES ON SOLID NUTRIENT MEDIUM TAKEN FROM HEPA-FILTER MEDIUM WITH AGAR CONTACT SAMPLING TECHNIQUE AFTER 26 MONTHS' OPERATION OF FILTERS

	Clean Air Side				Dust-laden Air Side			
Type of incubation	Number of probes	Filter surface investi-gated	Number of probes	Colony count per m^2 of filter surface	Number of probes	Filter surface investi-gated	Number of probes	Colony-Count per m^2 of filter surface
Aerobic	102	$6.5m^2$	3	<1	55	$1.6m^2$	254	156.41
Anaerobic	94	$6.2m^2$	0	0	51	$1.6m^2$	150	96.58

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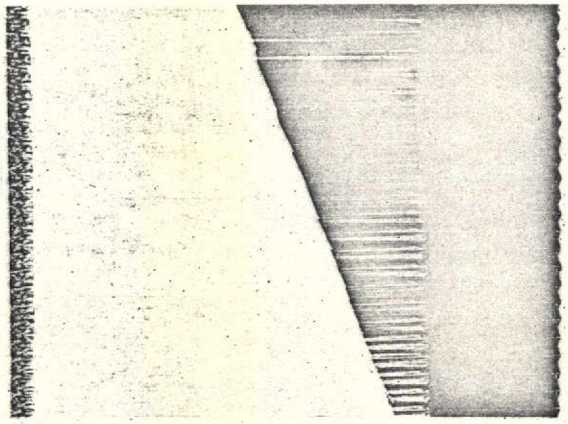

Figure 3. View of part of dismantled HEPA-filter after 26 months' operation of filter. To demonstrate the different degree of soiling by particulate matter at clean side and dustladen side of filter medium, the filter was taken apart and unfolded. The separator was cut transversally.

Of the total of 106 probes extracted from the dustladen air side of the filter medium for the sake of comparison, all showed a bacterial colonization(Table 3). The germ count, which is surprisingly low at first sight, can on the one hand be explained by the pre-filtering of the air which has already taken place; in addition, investigations of Botzenhart and Ruden [3] have shown that a large part of the germs which have been held back on HOSCH filters dies after a short period of time, and that hence one may not count upon an increase in the precipitating germs.

The germ counts determined by us, for comparison, on the clean air side of the pre-filters (after 2 months operation time) were on the order of magnitude of >800 germs per 50 cm^2 filter area with the predominant part being mould fungi; the precise counting of the colonies was often made difficult or impossible through formation of patches or colony superposition. /279

The gross differentiation of the isolated micro-organisms based on cultural and microscopic features is represented in

Table 4. The three germs isolated on the clean air side of the filter halves were Staph.albus.

TABLE 4.
QUALITATIVE DISTRIBUTION OF MICROBIAL SURFACE CONTAMINATION ON HEPA-FILTER MEDIUM AFTER 26 MONTHS' OPERATION OF FILTER

Number of Probes	Clean Air Side 196	Dust-laden Air Side 106
% positive probes	1.5%	100%
Number of isolated colonies	3	404
Gram-positive cocci		
hemolyzing	0%	0%
without hemolysis	100%	6%
Gram-positive small rods		
sporiferous	0%	59%
non-spore	0%	13%
Gram-negative small rods	0%	2%
Fungi	0%	20%

The main portion of the microorganisms isolated on the dust-laden air side of the filter medium consisted of sporiferous gram-positive small rods (59%). Fungi were represented at 20%, non-spore gram-positive small rods were represented at 13%. The portion of gram-positive cocci amounted to 6%, whereas gram-negative small rods were found in 2% of the isolated colonies.

Discussion

The question, which has been brought up often in recent times, of the "growing through" or "breaking through" of microorganisms on HOSCH filters has caused us to investigate the HOSCH filters of specialty class S which have been installed for 26 months in our air conditioning plant, for possible germ penetration.

As far as we know from the literature, the first indication that the microorganisms which colonize on the crude air side grow through the filter medium to the clean air side and can be pulled along with the stream of air came from Grun [7], who had made this observation on the precipitation of moisture on filters. According to investigations of Botzenhart and Ruden [2,3], however, a growing through of microorganisms is not to be expected for a relative humidity of up to 70%.

For the filtering of air, which in modern air conditioning plants is done in several stages, the last filter stage, for example, should be equipped with a filter of class S which is located on the pressure side immediately at the end of the air /280
canal inside the rooms to be ventilated [1,8,10,12,13,17,18]. This filter stage should be positioned so that depositing of water or a constant humidity over 70% is avoided, even when using the non-optimal spray humidification. A collecting of moisture at the air canal, at the filter mountings, or at the filters themselves has never been seen here, even for a temperature drop beneath the condensation point. The air which is brought to the HOSCH filters never exceeds a relative humidity of 65% here, in spite of spray humidification. In a normal case it amounted to 55-60%. In addition to a humidity that is too high, as a cause for the "growing through" of microorganisms through filters, in this connection one should also think of the correct drip-free construction of the filter holders in the mountings and of the intactness of the filter medium. For the drip test of our HOSCH filters with the particle counter which are installed and ready for work, we found strongly raised particle counts in the exiting air at one of the 27 installed HOSCH filters in Laboratory I, without noticeable macroscopic damage to the filter and the filter medium. In contrast to the particle

counts obtained in Figure 2, in HOSCH-filtered air in intact filters, the following particle counts were registered at a spot about 0.5 cm large at the jointing edge of a filter in the exiting air: 11,900 particles/m^3 of 0.5μ, 1300 particles/m^3 of 1μ, 140 particles/m^3 of 2μ, and 0 particles/m^3 of 5μ and larger. It is imaginable that a germ-break through would have occurred at this spot on the filter if the drip had been ignored. In addition, we carried out water-proofing probes with a smoke stone located in the main supply air canal, especially to test the setting of the filter holdings in the air canal nozzles. The investigations show that it is necessary and possible to undertake drip tests on the filters in situ before setting the plant free for operation.

We also routinely investigated the HOSCH-filtered air with a particle counter (CLIMET CI 250) and biological methods of measurement (slit-sampler, sedimentation plates) during the following 26 month time of operation of the air conditioning plant, in order to recognize and stop possible germ penetrations or non-watertightness in time. In the comparative particle and germ counts we have never been able to demonstrate germs during the time of operation, whereas on the average, 76 particles of the order of magnitude of 0.5μ, 5 particles of 1μ and 0 particles of 2μ and larger were measured. We realize that only an orientable value is to be subscribed to the measurements with the particle counter, which cannot replace germ counts. According to theoretical considerations by Frauch [6], one can count on the absence of bacteria if dust particles of 0.3μ and larger are absent. Muller [12] carried out simultaneous particle and germ counts in sterile OP-cabins and in the process ascertained that bacteria almost exclusively associated with particles that are larger than 5μ. Kethley [9], on the other hand, showed in his investigations that the large difference between biocontamination and particle count makes the installation of optical and mechanical methods for evaluating the biocontamination not seem suitable. Also, Wanner and co-workers [21], in comparative measurements of the particle and germ content of the air, established that the results depend strongly on the respective microbial sources of scattering. We did not undertake any bacteriological investigations on the filters themselves during the running operation, since the danger of mechanically damaging the filter medium with the manipulations connected therewith is present. In an orienting /281 experiment just before dismantling the filters, we artificially and superficially damaged the filter medium on the clean air side on an area of about 2 cm^2 and we found particle counts up to 240,000/m^3 of air, of 0.5μ and larger.

In spite of the massive impingement on the HOSCH-filters by particulate elements on the dust-laden air side, after a 26 months' operation time, culturally relatively few bacteria and fungi were demonstrable (Tables 3 and 4). A recalculation for the total filter area of a HOSCH filter (23 m^2) only yields a germ count of about 3000 germs per unit filter. To what extent a repeated blowing through of gas through the filter system during the 26 months' operation would have brought about a germ reduction, we have not been able to investigate, since this was not possible due to the occupation of the rooms with animals. Hence no disinfectant residues were found in the filter medium upon probe extraction.

We explain the low germ content on the one hand by the effective pre-filtering of the air, as we have already demonstrated in earlier investigations [14]. In addition, it cannot be excluded that small amounts of salt aerosol, torn along with the stream of air, impinge on the filter medium from the water of the spray moisturizing plant, and act as obstructing material, since the water at the present time still is prepared by a softening plant. The fine grained precipitate which was observed on the filter on the dust-laden air side was about 75% water soluble; the analysis yielded Na^{+}, Ca^{++}, Cl^{-}, SO_4^{--} ions, whereas carbonates were not demonstrated. Of the gram-negative germs frequently isolated in earlier investigations [14] behind the drop separator of the washing room, none could be found on the filter medium.

A direct comparison with the higher germ count values obtained by Botzenhart and Ruden [3] is not possible. The values determined by them were obtained by contact sampling techniques, which were taken from the exiting air side of HOSCH filters of a "pure work bench".

The results of the investigations, however, let us recognize that a large part of the germs held back on the filter quickly dies off on the filter medium, and reproduction does not take place. Similar statements are also made by McDade and co-workers [11] in investigations of the "plateau effects" on V_2A strips in bio-pure rooms.

The germs which have been demonstrated, very isolated, on the clean air side of HOSCH filters (Tables 3 and 4) may be viewed as contamination upon probe extraction. The fact that it is a matter of Staph.albus in the three isolated colonies out of 196 probes also speaks in favor of this. The use of enriched nutrient substrates or longer time of contact of the filter strips on the agar did not yield any higher yield of germs.

The results of our investigations of the HOSCH-filtered supply air and the filter medium did not permit us to recognize any penetration of germs under the climatic conditions here. It may, however, not be excluded, as for example in the case quoted by Grun [7], that mistakes at the air conditioning plant as well as a lack of hygenic technical maintenance and supervision can be the reason for germ penetrations. Looking for possibilities of lessening the danger of microbial penetrations through the filters, Eckstein [4] and van der Smissen [17] reported, at the International Symposium for Clean Room Technology in Zurich, 1972, on experiments to de-germ filters with UV radiation and ozone treatment. In the meantime, germ filter plants are being /282 offered by the Draeger Works, which are said to prevent germs on HOSCH filters by means of intensive UV radiation.

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